Our unborn children at risk?

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The study by Ang *et al.* (1) in this issue of PNAS strikes a precautionary note, in that it presents experimental evidence that prolonged continuous ultrasound (US) exposure may cause mild disruption in neuronal migration to the cerebral cortex in fetal mice. The authors (1) suggest that such neuronal heterotopia may result in anomalies in brain circuitry and synaptic activity. Does this study indicate that we should be concerned about human fetal US?

First, we will review the essential details of the study by Ang *et al.* (1). In the mouse, cells of the neocortex are formed and largely complete their migrations from their place of origin in the ventricular zone (VZ) to the cortex during the final week of a 19-day gestation (2). In these experiments, cells completing their final round of division on the 16th day of gestation, corresponding to the time of origin of cells of the outermost layers, were labeled by exposure to BrdU. A total of 146 embryos were exposed transabdominally *in utero* to a variable dosage schedule of B-mode US [unanesthetized dams; 6.7 MHz; 0.2-μs US pulse duration; 11 frames per second scanning rate; continuous exposure for between 5 and 35 min in graded schedules for a total exposure of 5, 15, 30, 60, 210, or 420 min; estimated attenuated spatial peak time average intensity (I_{SPTA}) of 0.6 mW per cm^2; spatial peak pulse average intensity (I_{SPPA}) of 262 W per cm^2; and mechanical index (MI), 0.66]. An exposure to US occurred on each of the final 3 days of gestation, that is, while cells arising on embryonic day 16 (E16) would be migrating. There were 141 sham control embryos and 30 control embryos.

The general appearance of the brains, examined on the 10th postnatal day, was unremarkable. There was no difference in brain size or neocortical cytoarchitecture and no histological evidence of tissue cavitation or other signs of tissue injury. In the sham control animals, the majority of neurons born on E16 were in layers II and III. In the experimental set, undergoing total exposures >30 min, a relatively small percentage of E16 neurons were located more deeply at all levels of the cerebral wall. At an exposure time of 30 min, a small subpopulation of heterotopic cells formed a discrete band in the subventricular zone (SVZ), just above the proliferative epithelium in the VZ. With increasing durations of exposure, heterotopic neurons became dispersed throughout the full width of the cerebral wall extending from the VZ through the cortical layers. The heterotopic neurons, including those lying in layer VI, did not stain with FoxP2, a marker for layer VI neurons, but some did stain for Brn1, a marker for outer-layer neurons. That is, with respect to these markers at least, they appeared to retain cell class-specific properties of neurons arising on E16 despite their abnormal positions.

Is the Experimental Model in the Mouse a Meaningful One for Human Fetal Biology?

The implications for the developing brain and its function are not known. First, the number of misplaced cells is small, so their effect may be little more than a minimal background noise factor. Second, the cells appear to retain their cell class characteristics despite malposition. If they are similar to the reeler mutation in mouse (3), where heterotopia is extreme, malpositioned neurons may not form connections that violate the rules of synaptic specificity. Third, after the completion of migration, a large fraction of neurons in all layers of all architectonic fields is normally eliminated by histogenetic cell death (4, 5), with neuron survival apparently contingent upon incorporation into adaptive circuitry (6–8). Thus, essentially all of the heterotopic cells in mice may be eliminated by histogenetic cell death. If heterotopia occurs in the human fetus as a consequence of US exposure, these heterotopic cells also may be eliminated by histogenetic cell death and would thus be of no consequence for the organization of the cortex. Finally, the migration of neurons formed on E16 in the mouse fetus would probably continue through E19 at the latest. The corresponding neurons in the human brain would probably be formed in the 16th week and continue to migrate for at least 1–2 weeks (9). Therefore, a proportionately larger fraction of the migration is exposed in the fetal mouse model. These uncertainties are clearly appreciated by the authors (1) and will serve as guides in their future experimental plan.

Did US Cause These Small Numbers of Neurons to Be Malpositioned?

It is a telling reflection of the complexity of the experimental model that, with an exposure of 420 min, there was heterotopic dispersion of more E16 neurons under sham control conditions than with any of the prior durations of exposure. That qualitatively similar patterns of heterotopia are found with the 420-min sham controls as are found with US suggests that mechanisms giving rise to heterotopia are not specific to US. Also, we note that although statistically significant differences were seen between fetal mice and sham controls with >30 min of exposure, there is uncertainty regarding the dose–response relationship in that the effect at 210 min of exposure was less than at 60 min of exposure. Thus, although the data provided here are compelling and appropriately cautionary, a dose–response relationship needs to be demonstrated in fetal mice.

If US Exposure Is Causing Heterotopia, What Is the Mechanism?

Sound waves are longitudinal waves of alternating high and low pressure. Diagnostic US is low-energy sound waves (usually between 1.6 and 10 MHz) that enter the tissue and reflect off of tissue interfaces. The reflected sound waves are detected and, because of their high frequency, allow us to create images with diagnostically sufficient levels of anatomic resolution. The bioeffects of US are most confidently ascribed to (*i*) thermal effects or (*ii*) mechanical pressure waves causing cavitation (10–13). The conditions under which heating and cavitation occur are not completely understood, but to minimize risks, the thermal index (TI) and mechanical index (MI) should be displayed in real time on all clinical US imaging equipment capable of exceeding a TI of 1 and an MI of 1 to alert the user to the potential risk of thermal or mechanical injury. In this study, the TI was not provided, but the I_{SPTA} (a measure of thermal effects) and MI were within U.S. Food and Drug Administration limits,

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although the I_{SPPA} was slightly high. Therefore, thermal and mechanical cavitation effects are unlikely to be the cause of heterotopia; the authors (1) emphasize that the mechanism causing heterotopia is not understood. Other known types of US bioeffects that may be the cause include acoustic streaming, propagation of shear waves resulting in torque, and radiation force (11).

How Well Matched to Routine Fetal US Is the Paradigm Itself?

The authors (1) underline the limits of comparability and offer cogent possibilities for differences, including the disparity in human-to-mouse species size and the associated vast difference in the relative size of cerebra in relation to the size of the US beam and exposure duration. We emphasize that these differences result in the fetal mouse paradigm being significantly different from routine fetal US. The weight of the fetal mouse brain at E16 is at most a few milligrams, whereas that of the human fetus in the sixth month is of the order of 100 gm, a difference of orders of magnitude. This extreme difference in brain size results in a significant difference in the volume of brain exposed to a US beam. In human fetal US, a slice of brain is imaged; the entire brain is covered in the plane parallel to the probe, but only a small fraction of brain is covered in the perpendicular direction. Because the fetal mouse brain is so small, this small spread of the US beam perpendicular to the scan plane is sufficient to include the entire brain. Moreover, for these experiments, the probe was held stationary for up to 35 min, meaning that essentially the entire fetal mouse brain would have been continually exposed to the US for 35 min. The continuous exposure of the entire brain in the experimental condition is in sharp contrast to the duration and volume of the human fetal brain exposed by US. Clinical fetal US studies adhering to the practice guidelines provided by the American Institute of Ultrasound in Medicine, in conjunction with the American College of Obstetricians and Gynecologists and the American College of Radiology (www.aium.org/publications/clinical/obstetrical.pdf), may have total scan times close to 30 min (14), but this includes the time required to survey the entire fetus: fetal presentation, amniotic fluid volume, cardiac activity, placental position, fetal size, and an anatomic survey of the entire body. In addition, this also includes the evaluation of the maternal cervix and adnexa. The total amount of time the US beam is directed upon the fetal brain is only a fraction of the total duration of the study. Furthermore, with human fetal US, the US beam is swept through the brain and has a width that is only a small fraction of the fetal brain. Therefore, because of the much-smaller relative size of the US beam, it will typically not linger on a given tissue volume for >1 min, well below the exposure time associated with heterotopia in this study.

Still, there can be no uncertainty about the capacity of US to have harmful effects. In the strongest terms, the findings of this study enforce the admonition that unregulated use of fetal US is to be avoided. Application of the principle of as low as reasonably achievable (ALARA) in US is recommended by practice guidelines and is a responsible guide for all fetal and pediatric imaging studies. This principle holds that the goal of a study is not an image with the maximal quality achievable but one that is sufficient to make a diagnostic judgment with the least possible exposure.

Are There Any Epidemiological Studies That Assess the Long-Term Effects of Fetal US?

Although large prospective randomized controlled trials have not been performed, there are many clinical indications where fetal US is believed to be beneficial (15). Reassuringly, however, a study recently published by Newnham *et al.* (15) based on prospective randomized controlled trials of exposure to US provides strong evidence that fetal US, as performed in an accredited clinical center, is unlikely to be linked to worst-case potential outcomes, such as developmental neuropsychiatric, epileptic, language, and cognitive disorders. Children exposed to five fetal US studies were compared at a followup of 8 years to a cohort exposed to only one fetal US. The estimated I_{SPPA} outputs were <5 mW per cm^2. Although a higher incidence of intrauterine growth restriction at 1 year was noted in the cohort with more US studies, this difference disappeared after the first year. In addition, it happened that children exposed to multiple scans actually performed better than controls in a language-acquisition task.

The Bottom Line

This study is a sober reminder that with US, as with all medical imaging, ALARA principles should be respected, and unregulated use of US should be avoided. US is capable of causing deleterious bioeffects to the human fetus, and the paper by Ang *et al.* (1) reminds us of the need to maintain our vigilance. More fundamentally, the study may illustrate a new consequence of US where we have little or no understanding of the mechanism. We consider the possibility that this may involve streaming and shear effects, but in reality, the cause has yet to be determined. However, given the marked differences in the volume of brain exposed and the duration of constant exposure, not to mention the biological differences between mice and humans, we view as highly unlikely the possibility that the present findings speak directly to risks of fetal US as currently practiced in competent and responsible centers. Moreover, applied appropriately, fetal US provides much benefit to prenatal surveillance.

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